Attorney's Docket No.: 10278-017001 / 0013

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Kinoshita et al.
Serial No. : 09/641,471
Filed : August 18, 2000
Title : HIGH MANNOSE PROTEINS AND METHODS OF MAKING HIGH MANNOSE PROTEINS

Art Unit : 1652
Examiner : Elizabeth Slobodyansky, Ph.D.

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF CAROL M. KINOSHITA, PH.D.

UNDER 37 C.F.R. § 1.132

I, CAROL M. KINOSHITA, pursuant to 37 C.F.R. § 1.132, declare the following:

1. My education and professional experience and qualifications are presented in the attached Curriculum Vitae (Appendix A).

2. I am a former employee of Shire Pharmaceuticals, which acquired Transkaryotic Therapies Inc. I am a named inventor on the above-referenced United States Patent Application No. 09/641,471.

3. I have reviewed the claims that are currently under examination in this application and understand them to recite methods of producing high mannose glucocerebrosidase (hmGCB) that includes a carbohydrate chain having at least four mannose residues. One method involves providing a mammalian cell that expresses a human glucocerebrosidase (GCB); contacting the cell with kifunensine; allowing the cell to produce hmGCB; and harvesting the hmGCB from the

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cell or its culture media. Another method involves providing a human cell into which a nucleic acid sequence comprising an exogenous regulatory sequence has been introduced such that the regulatory sequence is operably linked to, and regulates the expression of, an endogenous GCB coding region; contacting the cell with a class-I mannosidase inhibitor such that the removal of at least one mannose residue distal to the pentasaccharide core of a precursor oligosaccharide of GCB is prevented; and allowing the cell to produce hmGCB.

4. I have reviewed the Office Action mailed December 29, 2005 regarding the above-referenced application and understand that the Examiner has rejected claims 105, 106, 109-128, 130, 131, 133-138, 184, and 185 as obvious in light of Friedman et al. (US Patent No. 5,549,892) and Smith et al. (US Patent No. 5,939,279); and claims 129, 139-142, 144-164, and 167-171 as obvious in light of Treco et al. (US Patent No. 6,270,989) in combination with Friedman et al. and Smith et al.

5. As an initial matter, I address the difference between remodeling mature complex oligosaccharides and preventing the processing of precursor oligosaccharides with a class-I mannosidase inhibitor. **These two processes are distinct and thus the terms cannot be used interchangeably.**

An example of the process is as follows. After a protein is synthesized, it can undergo post-translational modifications to add oligosaccharides to the surface of the protein. For GCB, such modification occurs at four amino acids whereby four oligosaccharides are added to GCB. The four oligosaccharides that are initially added to GCB are all precursor forms that contain three glucose residues, nine mannose residues and two N-acetylglucosamine residues. Each of these precursor forms is then processed into one of three forms: a high mannose oligosaccharide, a hybrid oligosaccharide or a complex oligosaccharide. Typically, the high mannose form contains seven mannose residues and two N-acetylglucosamine residues; the hybrid form contains five mannose residues, three N-acetylglucosamine residues, and a galactose residue, and can optionally include sialic acid residues, fucose residues, etc.; the complex form contains at least three mannose residues; four N-acetylglucosamine residues, two galactose residues, and can optionally include sialic acid residues, fucose residues, etc. The form that the precursor is

processed into depends on the cell type producing the GCB. For example, as described in Friedman, the placental form of GCB has three complex oligosaccharides and one high mannose oligosaccharide, while the CHO cell-produced recombinant GCB has four complex oligosaccarides.

Processing of a precursor oligosaccharide (with nine mannose residues) typically occurs as follows: first, the three glucose residues are removed. Second, a class-I mannosidase removes up to four mannose residues resulting in an oligosaccharide that contains five to eight total mannose residues. Next, new sugars are added to the oligosaccharide, for example, more N-acetylglucosamine residues can be added. Fourth, class-II mannosidases remove additional mannose residues, resulting in an oligosaccharide that contains only three mannose residues and the two original N-acetylglucosamine residues. This structure, which contains three mannose residues and two N-acetylglucosamine residues, is termed the "pentasaccharide core."

If an oligosaccharide is destined to become a complex chain, two N-acetylglucosamine residues and two galactose residues are added to the pentasaccharide core. Additional residues such as sialic acid, fucose, etc. can also be added. Additional branching to form tri- and tetra-antennary structures can also occur.

After a precursor oligosaccharide (containing nine mannose residues) has been processed to a mature form (a high mannose form, a hybrid form, or a complex form), the mature form of the oligosaccharide can be remodeled in vitro, i.e., digested with enzymes to remove sugar residues and/or other groups (such as sialic acid groups) from the oligosaccharide. For example, a complex oligosaccharide can be treated with a neuraminidase to remove sialic acid residues, with galactosidase to remove galactose residues, with β–N-acetylglucosaminidase to remove N-acetylglucosamine residues. If all three of these enzymes were used, the oligosaccharide would be remodeled to the pentasaccharide core because all of the other groups would have been removed from it. These enzymes can only act on residues that are terminal, e.g., exposed on the oligosaccharide at the reducing end. Thus, the enzymes act sequentially. For example, if the sequence of moieties on an oligosaccharide is (starting from the reducing end of the oligosaccharide): sialic acid, galactose, N-acetylglucosamine; then the remodeling enzymes would act in the following order: neuraminidase to remove sialic acid, galactosidase to remove

galactose residues, and then β–N-acetylglucosaminidase to remove N-acetylglucosamine residues.

Thus, in summary, the building and remodeling of a complex oligosaccharide typically involves the following steps: 1- a precursor oligosaccharide is added to a protein, 2- the precursor is processed by removal of the glucose residues, 3- and by removal of the class-I mannose residues, 4- new sugars are added to the oligosaccharide, 5- more processing occurs by the removal of class II mannose residues, resulting in a pentasaccharide core, 6- new sugars, such as fucose (and optionally, other moieties such as sialic acid) are added to the pentasaccharide core to create a complex chain, 7- for remodeling, the complex chain is sequentially digested with enzymes specific for groups present at the reducing end of the complex chain, resulting in a remodeled complex form.

In sharp contrast, preventing the processing of a precursor oligosaccharide with a class-I mannosidase inhibitor is a dramatically different process. Simply put, this keeps all but the first two steps described above from happening. To prevent precursor processing, a class-I mannosidase inhibitor, such as kifunensine, is added to cell culture. Assuming complete class-I mannosidase inhibition, the only processing step that occurs after a precursor oligosaccharide is added to a protein is the removal of the three glucose residues that were present on the precursor. All subsequent steps of processing are blocked by the class-I mannosidase inhibitor. Thus, a protein synthesized in the presence of a class-I mannosidase inhibitor will contain oligosaccharides that contain the precursor chain's structure, i.e., the precursor's mannose residues will be on the chain. No complex or hybrid oligosaccharides are formed. Thus, no oligosaccharide that can undergo the above described remodeling is formed.

In summary, when the processing of a precursor oligosaccharide is prevented with a class-I mannosidase inhibitor, the only processing step that occurs is removal of glucose residues. Processing of the precursor oligosaccharide is not completed and no mature oligosaccharides are formed.

Thus, remodeling of a mature complex oligosaccharide and preventing the processing of a precursor oligosaccharide are distinct, dramatically different processes.

6. Regarding claims 105, 106, 109-128, 130, 131, 133-138, 184, and 185, I have read the Friedman and Smith patents and it is my opinion that based upon their disclosures, a skilled practitioner would not have been motivated to combine these references to practice the methods recited in these claims.

The Friedman patent compares remodeled placental (p-GCB) and remodeled recombinant (r-GCB) forms of GCB. Friedman concludes that remodeled r-GCB is targeted to Kupffer cells more effectively than is remodeled p-GCB. Friedman point out several differences between the two remodeled forms that could be responsible for the more effective targeting of r-GCB. These differences between remodeled p-GCB and remodeled r-GCB include: r-GCB possessing more fucose residues, r-GCB having four complex chains that underwent remodeling while p-GCB had only three complex chains that underwent remodeling, and an amino acid difference at residue 495: histidine in r-GCB versus arginine in p-GCB. Thus, at least two of the reasons Friedman suggest for more effective targeting of r-GCB highlight the importance of complex chains, namely the presence of fucose, and that a higher number of complex chains (versus chains with high mannose content) are important for more effective targeting after remodeling.

Significantly, the Friedman reference is a study of remodeled forms of r-GCB and p-GCB. As these forms were remodeled, they underwent the seven steps described in paragraph 5 above. There is no mention at all in Friedman about preventing the processing of a precursor oligosaccharide with a class-I mannosidase inhibitor. Thus, because Friedman only offers a comparison between r-GCB and p-GCB, it is my opinion that a skilled practitioner reading Friedman would be motivated to remodel mature forms of r-GCB as opposed to mature forms of p-GCB, because Friedman teaches that such remodeling of r-GCB improves targeting to cells of interest, namely, Kupffer cells.

The Smith patent is related to mechanisms of infection by gram-negative bacterial (such as Enterobacter bacteria). Because this reference is concerned with bacterial infections and has nothing to do with the production of mammalian proteins, it is my opinion that a skilled practitioner in the field of recombinant protein production would not read this patent as part of their professional activities.

The Smith patent teaches that Enterobacter bacteria bind to high mannose structures on the surface of mammalian cells as a first step in infection and that administering glycopeptides

containing nine mannoses to cell cultures inhibits bacterial adhesion to the cells. To confirm their model, Smith grew mammalian (HT-29) cells in the presence of kifunensine, a class-I mannosidase inhibitor, so that proteins on the cell surface would have high mannose structures. This resulted in increased bacterial adherence to the cells, lending further support to Smith's model of how bacteria infect cells.

A skilled practitioner would have concluded that Smith only showed that kifunensine treatment increased the amount of mannose present on the cell surface. However, GCB is present in the lysosomes of cells or is secreted. Smith does not give any indication that kifunensine treatment would have increased the mannose content of intracellular (i.e., lysosomal) and secreted proteins. Smith only dealt with cell surface proteins.

7. It is my opinion that even if a skilled practitioner in the field of protein production read both the Friedman and Smith references, this combination of references would not have motivated the skilled practitioner to culture cells that produce GCB in the presence of a class-I mannosidase inhibitor such as kifunensine.

The methods of Friedman and Smith cannot be combined because the two methods cannot operate together. Only a mature oligosaccharide can be remodeled, thus, a precursor oligosaccharide must first be processed to a mature (e.g., complex oligosaccharide) form before any remodeling can occur. The residues on the mature oligosaccharide are then sequentially removed by remodeling enzymes, as described above. However, if the processing of a precursor oligosaccharide to a mature oligosaccharide is prevented, e.g., by treatment with a class-I mannosidase inhibitor, no mature oligosaccharide is formed. Because no mature oligosaccharide is present, no substrates of the remodeling enzymes are present. For example, as described in Friedman, the enzymes used to remodel GCB included neuraminidase to remove sialic acid residues, galactosidase to remove galactose residues, and β-N-acetylglucosaminidase to remove N-acetylglucosamine residues. These residues are not present as terminal residues in a precursor oligosaccharide. Thus, the remodeling method of Friedman cannot work on an oligosaccharide synthesized in the presence of a class-I mannosidase inhibitor.

8. Regarding claims 129, 139-142, 144-164, and 167-171, I have read the Treco patent and it is my opinion that the Treco, Friedman, and Smith patents would not have motivated a skilled practitioner to practice the methods recited in these claims.

The Treco patent describes methods for increasing the levels of expression of three proteins: protein thrombopoietin (TPO), DNase I, and β-interferon and is not at all related to affecting post-translational modifications of proteins. Indeed, there is no mention at all about post-translational addition of oligosaccharides to proteins. As such, it is my opinion that in combination with Friedman and Smith, it does not provide any motivation to practice the methods recited in the claims, that is, to prevent processing of precursor oligosaccharides with a class-I mannosidase inhibitor. Also, as described above, because the methods described in Friedman cannot be applied to a precursor oligosaccharide, the methods of Friedman and Smith do not work together, and Treco does not solve this inoperability.

9. Finally, it is my opinion that the success of the methods described in the claims was unexpected. Kifunensine is toxic to cells, and the idea of culturing cells in the presence of this inhibitor was met with skepticism. Inhibition of class-I mannosidase by kifunensine would inhibit complex and hybrid type glycan chain formation for all glycoproteins produced in the cells, some of which would presumably be needed for the cells' viable operation. In fact, problems with low cell viability were encountered and dealt with in the development of the manufacturing process for hm-GCB. Indeed, I was surprised when GCB was produced and secreted by cells grown in the presence of kifunensine and that the titer and duration of GCB production by these cells were adequate to support a manufacturing process intended for commercial production of hm-GCB.

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States

Code, and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date: June 29, 2006

Carol M. Kinoshita

Carol M. Kinoshita, Ph.D.

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Curriculum Vitae

Carol M. Kinoshita

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Experience:

Shire Pharmaceuticals, Cambridge, MA
August 2005 to May 2006: Senior Scientist II, Purification Process Development
Major Accomplishments:
Led efforts to develop a purification process for one of Shire's protein products from a new cell culture process.
Responsibilities:
Continued in the same role after Transkaryotic Therapies' acquisition by Shire Pharmaceuticals

Transkaryotic Therapies, Inc., Cambridge, MA
January 2002-July 2005: Senior Scientist II and Group Leader, Purification Process Development
January 2000-December 2001: Senior Scientist II, Protein Chemistry
January 1998-December 1999: Senior Scientist, Protein Chemistry
November 1992-December 1998: Scientist, Protein Chemistry
Major Accomplishments:
As CMC team leader, spearheaded the effort to manufacture clinical material for Phase I/II clinical trials for one of TKT's protein products.
On the purification front, played a key role in bringing one of TKT's protein products from initial research to commercial manufacturing and a second product to Phase I/II manufacturing.
Responsibilities:
Served as CMC team leader and spearheaded the process development and manufacturing efforts for one of TKT's protein products
Trained and supervised a group of 4 to 6 people in protein purification and characterization for 3 or more protein products in varying stages of development.

Department of Biology, Massachusetts Institute of Technology, Cambridge, MA
April 1990-November 1992: Postdoctoral Fellow
Major Accomplishments:
Used anchored PCR to characterize newly discovered mouse immunoglobulin light chain genes.
Employed mouse genomic Southern blotting to survey the lambda light chain genes in a wide variety of wild mouse species with regard to the newly discovered lambda light chain genes.

Department of Immunology/Microbiology, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL
March 1986-December 1988: Postdoctoral Fellow
Major Accomplishments:
Characterized the calcium-binding sites of C-reactive protein and the related protein, serum amyloid P component, by monoclonal antibody mapping, Scatchard analysis, and partial proteolysis.

Education:

1986 - Ph.D. in Nutritional Sciences; Minor in Biochemistry, Physiology, and Toxicology
University of Wisconsin
Madison, WI
1981 - M.S. in Nutritional Sciences; Biochemistry emphasis
University of Hawaii
Honolulu, HI
1979 - B.S. in Nutritional Sciences; pre-medical/research curriculum
Universiy of Hawaii
Honolulu, HI

Areas of Expertise:

Protein Purification and Characterization:
HPLC and conventional chromatography, polyacrylamide gel electrophoresis, isoelectric focusing, peptide mapping, partial proteolysis to study structure-function relationships, glycoprotein characterization.
Immunology: Western blot, dot blot, ELISA, monoclonal antibody mapping.
Molecular Biology: Polymerase chain reaction, cloning, DNA sequencing, Southern blot, agarose gel electrophoresis.

Publications/Abstracts:

Patents
United States Patent 6,083,725. Transfected Human Cells Expressing Human α-Galactosidase A Protein.
United States Patent 6,395,884 B1. Therapy for α-Galactosidase A Deficiency.
United States Patent 6,458,574 B1. Treatment of α-Galactosidase A Deficiency.
Full Papers
Kinoshita CM, Lichton IJ, Ako H: A 50,000 dalton anorexigenic protein from urine. *Biochim Biophys Acta* 719:232-237, 1982
Biermann CJ, Kinoshita CM, Marlett MA, Steele RD: Analysis of amino acids as tert.-butyldimethylsilyl derivatives by gas chromatography. *J Chromatogr* 357:330-334, 1986

Kinoshita CM, Ganther HE: Isolation of a novel testicular metalloprotein binding cadmium and zinc. *Biol Trace Elem Res* 17:189-206, 1988

Potempa LA, Zeller JM, Fiedel BA, Kinoshita CM, Gewurz H: Stimulation of human neutrophils, monocytes and platelets by modified C-reactive protein (CRP) expressing a neo-antigenic specificity. *Inflammation* 12:391-405, 1988

Kinoshita CM, Ying SC, Hugli TE, Siegel JN, Potempa LA, Jiang H, Houghten RA, Gewurz H: Elucidation of a protease-sensitive site involved in the binding of calcium to C-reactive protein. *Biochemistry* 28:9840-9848, 1989

Ying SC, Gewurz H, Kinoshita CM, Potempa LA, and Siegel JN: Identification and partial characterization of multiple native and neoantigenic epitopes of human C-reactive protein using monoclonal antibodies. *J Immunol* 143:221-228, 1989

Kinoshita CM, Gewurz A, Ying SC, Siegel JN, Gupta R, Huckman R, Gewurz H: A protease-sensitive region involved in the binding of calcium to human serum amyloid P component and other pentraxins. *Protein Science* 1:700-709, 1992

Reidl LS, Kinoshita CM, and Steiner, LA: Wild mice express an immunoglobulin V lambda gene that differs from any V lambda in Balb/c but resembles a human V lambda subgroup. *J Immunol* 149:471-480, 1992

Theses

Kinoshita CM: Purification of a 50,000 dalton anorexigenic protein from rat urine. Masters thesis, University of Hawaii, 1981

Kinoshita CM: Purification and characteristics of the 30,000 dalton Cd and Zn-binding protein from rat testis cytosol. Ph.D. thesis, University of Wisconsin, Madison, 1986

Abstracts

Kinoshita CM, Lichton IJ, Ako H: Identification of a 50,000 dalton anorexigenic protein in rat urine. *Fed Proc* 41:1003, 1982

Kinoshita CM, Ganther HE: Purification of the 30 kilodalton metal-binding protein from rat testis cytosol. *Fed Proc* 44:1426, 1985

Kinoshita CM, Gewurz H: Proteolysis of C-reactive protein and serum amyloid P component by various proteases. *Fed Proc* 46:397, 1987

Kinoshita CM, Siegel JN, Potempa LA, Gewurz H: The effect of calcium-inhibited partial proteolysis of human C-reactive protein on calcium-dependent binding reactivity. *FASEB J* 2:A1149, 1988

Gewurz A, Kinoshita CM, Ying SC, Gupta R, Eatman J, Gewurz H: Characteristics of the binding of serum amyloid P component to chromatin. *FASEB J* 2:A1180, 1988

Ying SC, Gewurz H, Kinoshita CM, and Siegel JN: Identification of multiple epitopes on native and neoantigenic human CRP using monoclonal antibodies. *FASEB J* 3:A1345, 1989

Affiliations/Organizations:
Chicago Association of Immunologists, 1986-1988
Society for Investigative Nutrition, 1987-1988
American Association for the Advancement of Science, 1986-present

Honors/awards:
Knapp Memorial Fellowship, 1984-1986
Wisconsin Alumni Research Foundation Fellowship, 1982-1983
Gamma Sigma Delta Agricultural Honor Society, 1980
Carey D. Miller Award, 1980
Food and Nutrition Faculty Award, 1977, 1980
Student Marshall for College of Tropical Agriculture, 1981
Bachelor of Science degree with distinction, 1979
Phi Kappa Phi Senior Honor Society, 1979
Nutrition Council Chairperson, 1978
Phi Eta Sigma Freshman Honor Society, 1976-1979
Alpha Lambda Delta Freshman Honor Society, 1976-1977